



Integrated biodiesel and biogas production from microalgae: Towards a sustainable closed loop through nutrient recycling

Lina María González-González^a, Diego F. Correa^a, Stephen Ryan^a, Paul D. Jensen^b, Steven Pratt^c, Peer M. Schenk^{a,*}

^a Algae Biotechnology Laboratory, School of Agriculture and Food Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia

^b Advanced Water Management Centre, The University of Queensland, Brisbane, Queensland 4072, Australia

^c School of Chemical Engineering, The University of Queensland, Brisbane, Queensland 4072, Australia

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ABSTRACT

The sustainable, efficient production of biofuel can lead to reductions in greenhouse gas emissions, lowered climate change impact and increased security owing to the fulfilment of global energy demands. Microalgae have been shown as an attractive feedstock for renewable fuel production, such as biodiesel and biogas. To date, more effort has been put towards the production of biodiesel using the lipid contents in algal cells, while less attention has been placed on biogas production through anaerobic digestion. However, anaerobic digestion has the potential to generate energy from waste residues and to mobilize nutrients enabling subsequent recovery and/or recycling. Therefore, anaerobic digestion is an area with strong potential for novel research focusing on the development of a sustainable integrated system of biodiesel and biogas production. The result is essentially a solar power plant, producing fuel with minimal inputs and a closed nutrient loop, a necessity for sustainable and cost-efficient production of biofuel. In this review we discuss relevant studies on biodiesel and biomethane production, including the potential improvements and advantages when using an integrated approach for biodiesel and biogas production with special focus on nutrient recycling.

1. Introduction

Biofuels have been considered a promising sustainable alternative for energy production, potentially decreasing the emission of greenhouse gasses. Currently, liquid biofuels are mainly produced in the forms of bioethanol and biodiesel from different agricultural feedstocks, including oil crops such as oil palm, soybean and rapeseed, and sugar and starch crops such as sugarcane and corn [1,2]. These biofuels (named first generation biofuels) have been shown to be unsustainable and insufficient to meet the increasing energy demands, due to uncertain/poor energy balances, high water demands and high nutrient requirements, as well as competition for arable lands and thus with food crops [3–7]. Second generation biofuels seem to be an interesting alternative since they are produced from non-food biomass, including agricultural wastes and ligno-cellulosic feedstock such as wood, grasses and forest residues [4,8]. However, their potential to sustainably satisfy world energy demands is a matter of debate, in terms of feedstock availability and potential negative effects on carbon balances and biodiversity [9,10]. On the other hand, biofuels produced from microalgal biomass (third generation biofuels) can potentially overcome the

drawbacks of first and second generation biofuels, being more productive and sustainable [11–13].

Microalgae have faster growth rates than other crops and thus higher yields per unit area. The selection of productive strains can lead to the harvesting of cells with high lipid and carbohydrate contents, and different strains can be grown in fresh, brackish and sea water. Microalgae cropping does not compete with food crops since they can be produced in non-arable land, and additionally can be grown in wastewater as their culture medium, reducing the use of freshwater and nutrients [11–15]. Microalgae as a source of biofuels have been widely studied for the production of bioethanol [16–19], biodiesel [20–26], biogas through anaerobic digestion [27–32] and biohydrogen [33–36]. However, more research is needed in order to increase the efficiency for microalgal biofuel production and thus enhance its commercial viability, and at the same time increase the sustainability of the production process.

Maximizing production and reduction of inputs could be achieved through the development of an integrated system for biodiesel and biogas production, using anaerobic digestion (Fig. 1). That is because, besides biogas production, anaerobic digestion leads to the production

* Corresponding author.

E-mail address: p.schenk@uq.edu.au (P.M. Schenk).

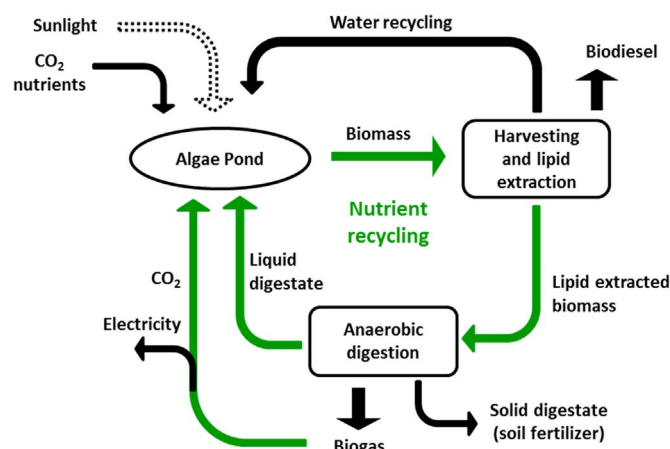


Fig. 1. Schematic model of an integrated closed loop of biodiesel and biogas production using microalgae. The diagram shows minimal inputs in an integrated system for solar biodiesel and biogas production. In this system, lipids are extracted from the concentrated biomass while water is reused to repeat algae cultivation. The defatted biomass is used as substrate for anaerobic digestion to produce biogas. Biomethane is burned to produce the electricity needed to maintain the system, while nutrients and CO_2 are recycled. The liquid phase of the digestate is used as algal culture broth and the solid phase can be used as soil fertilizer. Nutrient recycling is highlighted in green color.

of an effluent that can be used as fertilizer for algae culture, reducing the need of costly nutrients [37]. Thus, the integration of biodiesel and biogas production through anaerobic digestion of algae debris after lipid extraction is a promising way to significantly enhance methane production [38–40], while the recycling of nutrients from anaerobic digestion is a key step to make microalgal biodiesel production sustainable and reduce overall production costs [19,41–45]. In fact, Sialve et al. conclude that coupling anaerobic digestion with biodiesel production is essential for microalgal fuels to be viable [43]. Additionally, if biogas is used for the production of heat or electricity, CO_2 will be available for algae cultivation while reducing production costs [12]. The integrated system has the potential to reduce energy consumption and reduce up to 71% greenhouse gas emissions compared to petroleum fuel [46].

Here, we wish to provide a perspective on a closed loop system focused on nutrient recycling, including an analysis on available pre-treatments for cell disruption that may enhance biofuel production. Through this review we aim to bring together relevant studies on biodiesel and biomethane production from microalgae, focusing on nutrient recycling through anaerobic digestion for the development of a sustainable and profitable closed loop for energy production with minimal inputs. First, we describe the current methods and requirements for the production of biodiesel and biomethane, comparing different microalgal strains in terms of biodiesel and biogas production potential. Then, we provide an overview of the different pre-treatments that can be suitable both for biodiesel and biogas production, which will potentially improve an integrated biorefinery. Finally we discuss the advances towards the development of an integrated nutrient closed loop for biofuel production through anaerobic digestion, including economic and sustainability aspects.

2. Algal biomass production: culture conditions and harvesting

Light and nutrients are the main factors that determine cell production while herbivory and sedimentation lead to population loss [47], becoming the main factors that should be controlled in order to guarantee algal productivity and consequently high biomass yields. Besides, other variables such as temperature, pH, turbulence and salinity are crucial for culture growth [12–14,48].

Different microalgae species have specific nutrient requirements and are limited by different resources [49,50]. Within inorganic

Table 1

Elementary composition of microalgae.

Source: Adapted from Healey [53] and Grobbelaar [54].

Element	Compounds	Cell composition ($\mu\text{g}/\text{mg}$ dry weight)	
		Average	Range
H	H_2O , organic molecules, H_2S	65	29–100
C	CO_2 , HCO_3^{2-} , CO_3^{3-} , organic molecules	430	175–650
O	O_2 , H_2O , organic molecules	275	205–330
N	N_2 , NH_4^+ , NO_3^- , NO_2^- ; amino acids, purines, pyrimidines, urea, etc	55	10–140
Si	$\text{Na}_3\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	54	0–230
K	Several inorganic salts, i.e. KCl , K_2SO_4 , K_3PO_4	17.3	1–75
P	Several inorganic salts, Na or K phosphates, $\text{Na}_2\text{glycerophosphate} \cdot 5\text{H}_2\text{O}$	11	0.5–33
Na	Several inorganic salts, i.e. NaCl , Na_2SO_4 , Na_3PO_4	6.1	0.4–47
Mg	Several inorganic salts, i.e. CO_3^{2-} , SO_4^{2-} or Cl^- salts	5.6	0.5–75
Ca	Several inorganic salts, i.e. CaCO_3 , Ca^{2+} (as chloride)	8.7	0.0–80
S	Several inorganic salts, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, amino acids	5.9	1.5–16
Fe	FeCl_3 , $\text{Fe}(\text{NH}_4)_2\text{SO}_4$, ferric citrate	5.9	0.2–34
Zn	SO_4^{2-} or Cl^- salts	0.28	0.005–1.0
B	H_3BO_3	0.03	0.001–0.25
Cu	SO_4^{2-} or Cl^- salts	0.1	0.006–0.3
Mn	SO_4^{2-} or Cl^- salts	0.06	0.02–0.24
Co	Vitamin B_{12} , SO_4^{2-} or Cl^- salts	0.06	0.0001–0.2
Mo	Na^+ or NH_4^+ molybdate salts	0.0008	0.0002–0.001

nutrients, macronutrients (mainly nitrogen and phosphorus) are needed at high concentrations—on average, the production of 1 kg dry algal biomass requires around 55 g N and 11 g P. On the other hand, micronutrients are needed at low concentrations and have a specific metabolic role on microalgae physiology [48,51,52]. The specific optimal proportion of nutrients for each species may change depending on factors that include growth rate, temperature, light or CO_2 availability. Likewise, species differ in their nutrient requirements and nutrient uptake kinetics which results in different optimal proportions [52]. A general list of the main nutrients required by algal cells is detailed in Table 1.

Because moisture content can reach more than 99% of total microalgae cultures [55], harvesting is one of the biggest bottle necks for biodiesel production. Harvesting is difficult due to the small size of microalgae, their low specific gravity and similar density of the growth medium. Microalgae typically form stable suspensions in the water column and their high growth rates require regular harvesting [55,56]. Many techniques for primary dewatering of microalgae have been developed (sedimentation, flocculation, flotation, filtration and centrifugation) but many of them are strain specific or have complicated operation methods that represent high economic and energy costs [15,56,57]. For achieving a sustainable and profitable integrated biofuel production system, the most efficient and economic harvesting method is sedimentation by gravity. In this way, the economic impact is minimal and ideally, wet biomass can be used for direct lipid extraction.

3. Biodiesel from microalgae

Microalgae have the capability to accumulate large amounts (20–50% dry weight) of triacylglycerides (TAGs)—which are the main compounds for biodiesel production—especially under nutrient deprivation, photo-oxidative stress or other disadvantageous environmental conditions [58,59]. Several studies have reported increases in lipid

Table 2

Lipid contents and lipid productivities of several microalgal strains.

Species	Culture conditions	Lipid content (% of DCW)	Lipid productivity (mg L ⁻¹ d ⁻¹)	Ref.
<i>Chlorella protothecoides</i>	Heterotrophic growth with hydrolysate of Jerusalem artichoke	43–46	1881.3–1840.0	[75]
<i>Chlorella protothecoides</i>	Heterotrophic growth with corn powder hydrolysate	55.2 ± 0.3	932	[76]
<i>Chlorella protothecoides</i> UTEX 255	Heterotrophic growth with KNO ₃	50.5	654	[77]
<i>Nannochloropsis</i> sp.	Combined conditions of salinity (13, 27 and 40 g/L NaCl), light intensity (170 and 700 µE/m ² s) and nitrogen availability	35–48	385–413	[78]
<i>Chlorella zofingiensis</i>	Heterotrophic growth with glucose	51.1	354	[79]
<i>Desmodesmus</i> sp.	Nitrogen starvation	53.8 ± 6.0	263	[80]
<i>Chlorella vulgaris</i>	Mixotrophic growth with 1% glucose	21 ± 1	254 ± 2	[72]
<i>Chlorella vulgaris</i>	Standard growth conditions	28.1 ± 4.3	204.9 ± 6.4	[81]
<i>Scenedesmus</i> sp.	Nitrogen starvation	12.6 ± 0.8	174	[82]
<i>Chlorella vulgaris</i>	1% glucose	23 ± 2	151 ± 3	[72]
<i>Nannochloropsis oculata</i>	2% CO ₂ aeration	29.7 ± 2.0	142	[67]
<i>Neochloris oleoabundans</i>	Sodium nitrate medium (5 mM)	34	133	[83]
<i>Chlorella</i> sp.	Urea limitation (0.1 g/L)	52.2	124	[84]
<i>Botryococcus braunii</i>	Standard growth conditions	45.0 ± 4.0	112.4 ± 11.5	[81]
<i>Botryococcus terribilis</i>	Standard growth conditions	49.0 ± 1.5	98.0 ± 3.4	[81]
<i>Scenedesmus obliquus</i>	Nitrogen deficient and nutrient deficient media	38.9	78.7	[85]
<i>Chlorella vulgaris</i> ESP-31	Photoheterotrophic growth with acetic acid feeding	50	78	[86]
<i>Ankistrodesmus falcatus</i>	Nitrogen, phosphorous and iron starvation	59.60	74.07	[87]
<i>Chlorella vulgaris</i> ESP-31	Mixotrophic growth with glucose and CO ₂	40–53	67–144	[88]
<i>Ankistrodesmus falcatus</i>	Standard growth conditions	16.5 ± 0.4	56.1 ± 1.8	[81]
<i>Nannochloropsis</i> sp. F & M-M24	Nitrogen starvation	30.9	54.8	[89]
<i>Chlorella</i> sp. BUM11008	Nitrogen starvation	42.8	54.0 ± 0.6	[90]
<i>Scenedesmus</i> sp. DM	Nitrogen starvation	21.1	53.9	[89]
<i>Chlorococcum</i> sp. UMACC 112	Nitrogen starvation	19.3	53.7	[89]
<i>Pavlova lutheri</i> CS 182	Nitrogen starvation	35.5	50.2	[89]
<i>Ankistrodesmus fusiformis</i>	Standard growth conditions	20.6 ± 2.1	49.6 ± 5.7	[81]
<i>Phaeodactylum tricornutum</i> F & M-M40	Nitrogen starvation	18.7	44.8	[89]
<i>Chlorella sorokiniana</i> IAM-212	Nitrogen starvation	19.3	44.7	[89]
<i>Tetraselmis</i> sp. F & M-M34	Nitrogen starvation	14.7	43.4	[89]
<i>Chlamydocapsa bacillus</i>	Standard growth conditions	13.5 ± 0.6	43.3 ± 2.4	[81]
<i>Chlorella</i> sp. BUM11010	Iron starvation	31.4	40.0 ± 0.8	[90]
<i>Chlorella</i> sp. BUM11009	Phosphate starvation	31.9	39.4 ± 0.5	[90]
<i>Isochrysis</i> sp. (T-ISO) CS 177	Nitrogen starvation	22.4	37.7	[89]
<i>Chlorella vulgaris</i> F & M-M49	Nitrogen starvation	18.4	36.9	[89]
<i>Scenedesmus quadricauda</i>	Nitrogen starvation	18.4	35.1	[89]
<i>Chaetoceros muelleri</i> F & M-M43	Nitrogen starvation	33.6	21.8	[89]
<i>Nannochloropsis</i> sp.	Nitrogen starvation	28.7 ^a	NR	[91]
<i>Neochloris oleoabundans</i>	Nitrogen starvation	29 ^a	NR	[91]

DCW = dry cell weight; NR = not reported.

^a Ash free dry weight.

contents by nitrogen or phosphorus starvation [24,60,61], UV stress [62,63], changes on temperature [64,65], irradiances [64,66], CO₂ concentration [67–69] and salinity [64,70,71], or under heterotrophic growth conditions [72,73]. Table 2 provides examples of lipid increments for different microalgal species under different stress conditions. Nitrogen starvation is the most widely used method to induce lipid accumulation, however, the optimum conditions to enhance lipid production will depend specifically of the strain, environmental conditions and cultivation system [74].

3.1. Lipid extraction and pre-treatments

After achieving high lipid productivities, the biomass is concentrated for lipid extraction. The recovered lipids are then transformed into biodiesel in the presence of catalysts through transesterification or hydrogenolysis conversion processes. These catalysts include alkalis (e.g. sodium methoxide, potassium hydroxide), acids (e.g. sulfuric acid, phosphoric acid), enzymes (e.g. lipases) or heterogeneous catalysts [91–94].

The extraction of lipids from algal biomass is a complex process in biodiesel production that can significantly increase production costs. Lipid extraction methods such as organic solvent extraction [95,96] or supercritical fluid extraction [97,98] require drying of the biomass. Dewatering is a complicated process that increases production costs and

reduces algal biofuel profitability. Additionally, lipid recovery is not 100% efficient. In order to improve lipid recovery percentages, and benefit the subsequent anaerobic digestion processes, several pre-treatments of the biomass have been developed (see Section 4.2). Pre-treatments provoke cell disruption and liberate the lipids inside the cells. In order to reduce lipid extraction costs, pre-treatments on wet biomass are preferable. These include hydrothermal liquefaction [99,100], microwave assisted extraction [101,102], enzymatic extraction [103,104], osmotic shock [21,105], oxidative stress [106], ultrasound assisted extraction [107,108] and pulsed electric field technology [109,110]. Here, we give a brief introduction to the most cost-efficient pre-treatments.

3.1.1. Hydrothermal liquefaction (HTL)

Hydrothermal liquefaction is an attractive method of biomass conversion which produces a relative stable oil product with low energy consumption in comparison with other conversion techniques [100]. Thermochemical conversion applies high pressure (10–25 MPa) and medium to subcritical temperature (below 374 °C) [99,111]. During the process, the biomass is hydrolyzed and degraded into small molecules and part of the oxygen in the biomass is removed by decarboxylation or dehydration [100]. With this technology, the energy recovery from biomass to fuel is around 80% [100] and less than 5% of the energy cost is required to complete the thermal drying [111].

Table 3
Methane production from the anaerobic digestion of different microalgae biomass.

Species	Reactor	T (°C)	Operation time (days)	Biogas yield (mL g ⁻¹ VS)	CH ₄ (mL g ⁻¹ VS)	CH ₄ (%)	Ref.
<i>Isochrysis</i> spp.	BMP test	35	34–50	NR	408 ± 4	NR	[148]
<i>Scenedesmus dimorphus</i>	BMP test	35	34–50	NR	397 ± 10	NR	[148]
<i>Scenedesmus</i> sp. (lipid extracted biomass)	BMP test	37	37.5	NR	393.6 ± 19.5	NR	[166]
<i>Chlamydomonas reinhardtii</i>	BMP test	38	32	587 ± 8.8	387.42	66	[28]
<i>Chlorella vulgaris</i>	BMP test	35	34–50	NR	361 ± 11	NR	[148]
<i>Porphyridium aeruginosa</i>	BMP test	35	34–50	NR	352 ± 3	NR	[148]
<i>Phaeodactylum tricornutum</i>	BMP test	34 ± 2	30	NR	350 ± 0.03	NR	[157]
<i>Euglena gracilis</i>	BMP test	38	32	485 ± 3	324.95	67	[28]
<i>Dunaliella salina</i>	BMP test	38	32	505 ± 24.8	323.2	64	[28]
<i>Tetraselmis</i> sp.	CSTR	35	NR	418.9–430.5	310	72–74	[151]
<i>Neochloris oleoabundans</i>	BMP test	35	34–50	NR	308 ± 1	NR	[148]
<i>Arthrospira platensis</i>	BMP test	38	32	481 ± 13.8	293.41	61	[28]
<i>Chlorella sorokiniana</i>	BMP test	35	34–50	NR	283 ± 4	NR	[148]
<i>Chroococcus</i> sp.	BMP test	36 ± 1	30	487 ± 16.73	267.36	54.9	[27]
<i>Thalassiosira weissflogii</i>	BMP test	35	34–50	NR	265 ± 15	NR	[148]
<i>Chlorella pyrenoidosa</i>	BMP test	36 ± 1	NR	464 ± 66	264.71	57.05 ± 0.89	[146]
<i>C. vulgaris</i>	CSTR	35	35	NR	240	NR	[150]
<i>Nannochloropsis gaditana</i>	BMP test	35	34–50	NR	228 ± 4	NR	[148]
<i>Glossomastix chrysoplata</i>	BMP test	35	34–50	NR	227 ± 8	NR	[148]
<i>Chlorella kessleri</i>	BMP test	38	32	335 ± 7.8	217.75	65	[28]
<i>Chlorella sorokiniana</i>	CSTR	40–41	71	248	212	85.48	[167]
<i>Scenedesmus obliquus</i>	BMP test	33 ± 2	30	NR	210 ± 0.03	NR	[157]
<i>C. vulgaris</i>	BMP test	36 ± 1	NR	369 ± 67	195.64	53.02 ± 0.46	[146]
Cyanobacteria mix	CSTR	35	NR	517.13	189.89	36.72	[152]
<i>C. vulgaris</i>	CSTR	40–41	110	221.1	189	85.48	[167]
<i>Scenedesmus obliquus</i>	BMP test	38	32	287 ± 10.1	177.94	62	[28]
<i>Chlorella minutissima</i>	BMP test	36 ± 1	NR	340 ± 114	166.12	48.86 ± 0.74	[146]
<i>C. vulgaris</i>	CSTR	35	65	NR	147	NR	[150]
<i>Arthrospira maxima</i>	CSTR	35	30	200	144	72	[32]
<i>Scenedesmus</i> spp. & <i>Chlorella</i> spp.	BMP test	35 ± 1	20	NR	143	NR	[30]
<i>Microcystis</i> spp.	BMP test	35	30	NR	140.48	35.92	[154]

NR = Not reported; BMP = biomethane potential; CSTR = continuously stirred tank reactor.

3.1.2. Oxidative stress

Free nitrous acid (FNA) is a low cost and effective pre-treatment that has been studied recently by Bai et al. [106] to improve lipid extraction through oxidative stress. The study reveals the efficiency of FNA in disrupting the cell membrane of algae cells which facilitates lipid recovery. The efficiency of this methodology increases with longer exposure time (48 h) and higher FNA concentration (up to 2.29 mg HNO₂-N/L). The authors report the highest total lipid extraction yield to be 2.4 fold higher on cultures treated with FNA compared with untreated biomass.

3.1.3. Osmotic shock

Osmotic shock is an abrupt change in osmotic pressure which causes the disruption of algae cells and the release of their cellular components [112], substantially improving lipid recovery. Yoo et al. [105] evaluated the effect of osmotic shock on lipid recovery from wet biomass of *Chlamydomonas reinhardtii*. Lipid recovery after osmotic shock was two times higher in comparison with untreated biomass. Besides, the study reveals the importance of the cell wall and growth phase on the efficiency of this method, concluding that senescent cell phase is optimal for lipid extraction. This is a suitable, easy, cheap and scalable technic for species with a thin or fragile cell wall.

4. Biogas from microalgae

Anaerobic digestion is a widely utilized process for treatment of organic wastes that leads to the production of methane-rich biogas. This is a complex process in which specialized microorganisms (hydrolyzing, fermentative, acetogenic, homoacetogenic, sulfate-reducing and methanogenic archaea) decompose organic compounds in an oxygen-free environment [113]. The microbial consortia work together to decompose complex organic substances into simple and chemically-stable compounds, such as methane and carbon dioxide through a series of

biochemical reactions, including hydrolysis, acidification, acetogenesis and methanogenesis [4,113,114]. Key variables that affect the performance of an anaerobic digestion process are substrate composition, water content, temperature, pH, alkalinity, organic loading rate and hydraulic retention time [114]. Biogas composition is impacted by both the substrate composition (which impacts methane and CO₂ ratios) and pH (which regulates the speciation of the carbonate system and CO₂ release) [115].

The study of anaerobic digestion for biogas production was initially concentrated on the biodigestion of different wastes such as wastewater [116–119], slurry [120–123], manure [124–127] or food waste [124,128–130]. Additionally, several studies have been reported on the anaerobic digestion of different crops, such as maize silage [131–135], straw [136–140] and grass silage [141–143].

Research in biogas production from microalgae has recently increased due to their advantages over other feedstocks. Microalgal biomass is a suitable substrate for anaerobic digestion since mineral composition of algae cells fits the nutrient demands of anaerobic bacteria [43]. The solar energy stored in algal cells is converted into usable energy by burning the methane liberated in the anaerobic digestion process [144]. Besides their fast growth and their capacity to produce high densities of biomass on a small and non-arable area, microalgae release less hydrogen sulphide than other substrates, due to their low amount of sulphurated amino acids [43].

The first study on anaerobic digestion of microalgae was published by Golueke et al. [144] who made a comparison between anaerobic digestion of green microalgae and raw sewage resulting in a similar methane yield. After this, many studies have been published focused mainly on the anaerobic digestion of green algae [28,145–151] and cyanobacteria [27,28,32,152–154]. Other algae groups that have been studied for biogas production are euglenophyceans [28,155], diatoms [156–158], and even macroalgae [29,159–161]. An overview of the biogas yield from different microalgal species without pre-treatments is

included in Table 3. The average percentage of biomethane in biogas is around 60% and the maximum methane yield reported to date is around 400 mL g⁻¹ Volatile Solids (VS) [28,145,163].

Although biogas production from microalgae has been mainly focussed on chlorophyceans (green algae) from the genera *Chlorella* [28,144,150,162,163] and *Scenedesmus* [40,144,148,157,164], these genera usually report low biomethane yields compared to other species due to their rigid cell wall that hinders the biodegradability of the cells [28,43]. In the comparative analysis of biogas production from different species developed by Mussnug et al. [28], *Chlorella kessleri* and *Scenedesmus obliquus* reported the lowest methane yields (218 and 178 mL g⁻¹ VS, respectively) while other chlorophyceans as *Chlamydomonas reinhardtii* (protein-based cell wall without cellulose) and *Dunaliella salina* (without cell wall) reported biomethane yields of around 387 and 323 mL g⁻¹ VS respectively. This suggests that microalgal species with thin cell walls are more digestible and should be preferred as feedstock for biogas production from anaerobic digestion. All of the studies reported in Table 3 did not perform pretreatments on microalgal biomass and higher yields can be expected if pretreatments are used to break the cell walls for increased digestibility (see Section 4.2).

Biogas yields from cyanobacteria species are generally lower compared to those obtained by chlorophyceans. Yuan et al. [152] reported a methane yield of 189.89 mL g⁻¹ VS following the anaerobic digestion of a mix of cyanobacteria mainly composed of the genus *Microcystis*. The average methane concentration in the biogas was only 36.72% while generally this percentage is around 60–75% with other microalgal feedstocks [43,165]. A lower yield with this genus was reported by Zeng et al. [154] who described a maximal methane yield of 140.48 mL g⁻¹ VS and maximal methane concentration of 45.19%. Samson and Leduy [32] reported a better yield following the biodigestion of *Spirulina maxima* (260 mL g⁻¹ VS), with an average methane concentration of 68–72%, while Mussnug et al. [28] obtained a biomethane yield of 293 mL g⁻¹ VS and 61% of methane concentration based on the digestion of *Arthrospira platensis*.

4.1. Limitations to anaerobic digestion of algae

In order to guarantee the financial viability of an optimal biogas yield it is very important to take into account some aspects that can restrict the efficiency of anaerobic digestion when using microalgae as substrate, such as cell wall degradability, ammonium toxicity and salinity.

4.1.1. Cell wall degradability

Algal cell walls of prokaryotic (cyanobacterial) and eukaryotic algae are composed of a rigid, homogenous and often multilayered structure whose chemical composition differs between different groups [48,168]. In general, algal cell walls contain two main components: 1) the fibrillar component which comprises the skeleton of the cell wall (generally cellulose), and 2) the amorphous component (composed of polysaccharides, lipids and proteins) where the fibrillar component is enclosed [48,169]. Some species of microalgae have a tight cell wall that can be highly resistant to anaerobic degradation which reduces the biodegradability of algae cells, resulting in a low biogas yield. Foree and McCarty [170] evaluated anaerobic decomposition of several microalgal substrates resulting in 41% of the initial particulate matter undecomposed after 200 days. Species with a thin or weaker cell wall can be more easily digested than those with a thick and rigid cell wall, such as that found in *Scenedesmus* sp. This was demonstrated by Mussnug et al. [28], who evaluated the anaerobic digestion of six different strains of microalgae. The species without a cell wall or those with a protein-based cell wall gave the highest biogas yields, due to the better digestibility of algal cells. The authors suggest that cell wall composition and the production of certain bactericidal compounds can be inhibitory factors that require the application of adequate pretreatments. In order to improve biogas yield of certain species it is

mandatory to implement pre-treatments or select species without cell walls [150].

4.1.2. Ammonia toxicity

The equilibrium between ammonium (NH₄⁺) and its un-ionised form ammonia (NH₃) is primarily mediated by pH and temperature. Any change in one of these variables could lead to liberation of ammonia which can become very toxic for the bacterial community (especially for methanogenic archaea) altering the good performance of the anaerobic digester [171,172]. As nitrogen is an essential nutrient for anaerobic microorganisms, total ammonia nitrogen concentrations below 200 mg L⁻¹ are beneficial for the anaerobic digestion process [173].

Anaerobic digestion of microalgae can deal with ammonium toxicity since generally their C/N ratios are low due to their high protein content. C/N ratios below 20 lead to ammonia liberation [171]. One way to deal with ammonia toxicity is to use co-digestion of microalgae with rich carbon compounds which also improves methane yield. Zhong et al. [174] reported an increase of 61.69% on the methane yield from blue algae sludge when co-digested with corn straw. The methane yield increased from 201 mL g⁻¹ VS with algae sludge substrate alone to 325 mL g⁻¹ VS after co-digestion. An increase of 66.4% in biomethane yield of *Scenedesmus* sp. was observed when co-digested with *Opuntia maxima* plants [145]. Similar results were obtained for the co-digestion of algae sludge and waste paper, which had a methane yield (1170 mL L⁻¹ day⁻¹) more than two-fold than from the algae alone (573 mL L⁻¹ day⁻¹) [30]. On the other hand, ammonia inhibition can be partially addressed through pH and temperature control as the ionised non-toxic form of ammonia (ammonium – NH₄) increases with pH below 7 and higher temperatures [114,171]. During microalgae cultivation the pH can be conveniently controlled by the addition of CO₂.

4.1.3. Salinity

Sodium toxicity may be an important inhibitor when the feedstock for anaerobic digestion is an algal culture of marine species. High salt concentrations in marine cultures can be very toxic for methanogenic archaea due to dehydration by osmotic pressure [171,175]. Besides, the use of NaOH or Na₂CO₃ for pH control can increase the sodium concentration [176]. Sodium toxicity on anaerobic digestion has been reported by many authors, but there is no consensus neither in the optimal sodium concentration nor in the toxic concentration since the inhibitory effect is different for each anaerobic community on the digester and the presence of other ions in each case may play an antagonistic role in sodium toxicity [176–178]. Patel and Roth [179] reported an optimal sodium concentration of 345 mg Na⁺ L⁻¹ for methanogens while Kugelman and Chin [180] suggested 230 mg Na⁺ L⁻¹ as the optimum for acetoclastic methanogens. An inhibitory effect at 2 and 6 g Na⁺ L⁻¹ was reported by Patel and Roth [179] but Rinzema et al. [181] reported an inhibitory effect of 10%, 50% and 100% at sodium concentrations of 5, 10 and 14 g Na⁺ L⁻¹, respectively, in acetoclastic methanogenic activity. Despite the different values, the inhibitory concentration seems to be quite far from its optimum which suggests an adapting potential to sodium toxicity. Salinity tolerance of methanogenic archaea has been shown to increase after an adaptation period instead of shock exposure [176].

Because of this potential inhibition factor, few authors have evaluated marine species for biogas production and mostly with macroalgal species [29,182–184]. However, some studies with microalgal marine species have shown acceptable biomethane yields [28,148,157].

4.2. Pre-treatments to enhance algae digestibility

An effective way to solve the limitations discussed above and enhance algae digestibility is to apply a determined treatment to the algal sludge before digestion. All pre-treatments are focussed on cell disruption which is the main factor that obstructs algae digestibility and

reduces biogas production potential. When implementing an integrated approach for concurrent biodiesel and biogas production (Fig. 1), a suitable pre-treatment to improve anaerobic digestion may be already in place from the oil extraction step. In fact, although this has not been studied simultaneously in the literature, the pre-treatments that are effective for improved oil extraction from microalgae are very similar to those that have been reported to be effective for improved anaerobic digestion.

Many types of pre-treatments for anaerobic digestion have been described in the literature [185–187], but the efficiency on microalgae has been poorly studied. Microalgae digestibility after pre-treatments has been mainly evaluated by biomethane potential (BMP) tests and further research is needed in pilot-scale reactors to determine the scalability of the technology [185]. A complete review on pre-treatments to improve biogas production from microalgae has been recently published by Passos et al. [185]. Here, we describe some generalities of the most used techniques.

4.2.1. Thermal pre-treatment

Thermal pre-treatment is the most commonly used method to prepare microalgal feedstock for anaerobic digestion. Under high temperatures, there have been reported increments in algae biomass digestibility up to 48–60% [162,188]. Comparing with other methods, thermal pre-treatment is a promising treatment that is scalable and the energy input is negligible compared to the benefits [189,190]. With the improvement of biomass digestibility after pre-treatment, biomethane production is always higher in comparison to untreated biomass. Passos and Ferrer [190] reported 70% increment on biomethane yield at relatively low temperatures (75–95 °C) with an energy gain around 2.7 GJ day⁻¹ after digestion of 1.5 L of the pre-treated biomass. At the same temperatures, Passos et al. [191] reported an increment up to 61% while a significantly increase of 285% was reported for *Nannochloropsis salina* at 120 °C [189]. Another approach was developed by Keymer et al. [40] by applying high pressure thermal hydrolysis (HPTH) to both raw algae and lipid-extracted residues. The pre-treatment increased methane yield by 81% and 110% for raw and lipid-extracted biomass, respectively.

4.2.2. Mechanical pre-treatment

The principle of mechanical pre-treatment is to apply a physical force to the biomass. For example, mechanical treatments on microalgal sludge can be implemented with ultrasound and microwaves. The ultrasound treatment consists in fast compression and decompression cycles of sonic waves generating the formation of microbubbles inside the cells [192], while with microwaves, short waves of electromagnetic energy (300 MHz to 300 GHz) lead to a fast water boiling point provoking the weakening or rupture of some hydrogen bonded structures [193]. The main disadvantage of this treatment is that it can be very expensive due to high electricity consumption [185]. Among mechanical pre-treatments, ultrasound is the most commonly used for microalgal digestion. Its effectiveness depends on the energy dose (as higher energy doses lead to a higher degree of disintegration) and the target species. For instance, Lee et al. [194] reported an increase on methane production of 230% based on the anaerobic digestion of *Hydrodictyon reticulatum* with an energy dose of 40 J mL⁻¹, while for *Chlorella vulgaris* 200 J mL⁻¹ improved biomethane production by up to 90% [195]. Microwave pre-treatment has been tested on microalgae mainly for lipid extraction [21,196]. There are a few studies reported in the literature for biogas production. Although this treatment has shown an increment on algal biomass solubilization of up to 800% [197], the improvements on biogas yield reported are lower (maximum 40–78%) in comparison with ultrasound pre-treatment [197–199].

4.2.3. Chemical pre-treatment

Chemical pre-treatments are based in the addition of alkali, acid or an oxidative agent, such as hydrogen peroxide prior to digestion. The

main disadvantage of this treatment is the potential toxicity of different by-products [200]. Additionally, despite a low energy demand, chemical pre-treatment can be very expensive to be scalable due to high chemical costs. There are only a few studies about chemical pre-treatments on algal sludge for biogas production, as biomass solubilization and increases in methane yields are very low compared to other treatments [185]. However, the combination of chemical and thermal pre-treatments has shown very good results. For instance, Bohutskyi et al. [201] found that chemical treatment with alkali was not effective while thermochemical pre-treatments resulted in the highest biomass solubilization with an increase in methane yields of 30% and 40% for *Chlorella* and *Nannochloropsis* respectively.

5. Nutrient mobilization

Through anaerobic digestion a portion of the nutrients that go into the process can be mobilized and recycled. For instance, Zhang et al. [37] reported that 40.7 g of nitrogen (74%) and 3.8 g of phosphorous (35%) were recycled for 1 kg (dry weight) of digested *Scenedesmus dimorphus* biomass. The study shows that nitrogen recovery is highly efficient after anaerobic digestion, but most of the phosphorus remains trapped in the solid phase of the digestate and is useless for microalgae cultivation. Therefore, options on how nutrients from the solid phase can be reutilized for microalgae cultivation should be explored. Besides this study, there are no reports in the literature about N and P distribution in the liquid and solid phase of digestate from anaerobic digestion of microalgae, which makes difficult to predict the fertilizer value of an algal digestate suitable for a closed loop of biofuel production. Nevertheless, the same pattern described by Zhang et al. [37] has been found for digestates from other substrates [202–207]. It seems that during anaerobic digestion the available fraction of phosphorus is reduced and P solubility is controlled by increasing P stability on the solid phase [208]. Since pH has a strong effect on phosphorus solubility, high pH values result in calcium or magnesium phosphate (struvite) precipitation as the chemical equilibrium in that case favours PO₄³⁻ over HPO₄²⁻ [209]. Besides, phosphate release seems to be influenced by inoculum/substrate ratio (ISR). Zeng et al. [154] found a strong correlation between orthophosphate release and ISR in the anaerobic digestion of *Microcystis* spp. The study shows a 34% decrease in the orthophosphate release rate when the ISR decreased from 2.0 to 0.5. The authors explain this phenomenon as an enhancement of the decomposition rate of algae cells due to additional anaerobic microorganisms which in turn liberate more intracellular phosphorus.

On the other hand, anaerobic digestion effluent is characterized by its high ammonium content. Nitrogen is mostly liberated in the digestate as ammonium, due to a very low denitrification rate in the anaerobic digestion process [210]. At high retention times (28 days) and 35 °C, a nitrogen mineralization efficiency of 68% was reported [150].

6. Nutrients recycling and CO₂ production, the final step to close the loop

6.1. Nutrients recycling, the final step to close the loop

As we discussed above, besides gas production, anaerobic digestion generates a digestate rich in nutrients that can be used as fertilizer [211–213] giving an additional value to the process. The production of high quality fertilizers is being explored through extraction of the nutrients concentrated on digestates [115]. High levels of N, P and K as well as some micronutrients on digestates [211] make them a suitable source of nutrients for algae culture. Particularly the liquid phase of the digestate, characterized by the presence of low amounts of solids and high nutrient contents, is a good alternative to replace costly chemical fertilizers. Meanwhile, the solid phase of the digestate may inhibit light penetration in the cultures. Besides, organic N remains in the solid fraction of digestate while the liquid fraction is rich in mineralized N

Table 4

Comparison of energy inputs and energy yields for different coupled systems for biodiesel and biogas production.

Combined lipid extraction and biogas production			
Calculated energy demand	Calculated energy yield	Notes	Ref.
99 GJ. Energy comes from sludge, waste paper and methanol	40 GJ as biodiesel 17 GJ as electricity from methane combustion 49 GJ as heat from methane combustion 67 GJ as organic waste	– Theoretical model based on biomass productivities of 15.1 g m ⁻¹ day ⁻¹ – Assumes no external CO ₂ and nutrient inputs	[45]
59.5–99.5 GJ. Energy comes from sludge, waste paper and methanol	40–241.3 GJ as biodiesel 13.8–17 GJ as electricity from methane combustion 49.3 GJ as heat from methane combustion 26.9–71.4 GJ as organic waste	– Comparison between <i>Chlorella vulgaris</i> , <i>Nannochloropsis</i> sp. and <i>Haematococcus pluvialis</i> – Theoretical model based on biomass productivities between 15.1 and 64 g m ⁻² day ⁻¹ – Assumes no external CO ₂ and nutrient inputs	[44]
0.222–0.669 GJ. Energy comes from nutrients, heat, electricity, methanol, etc.	0.037–0.086 GJ as biodiesel 0.025–0.051 GJ as biogas	– Comparison between <i>Haematococcus pluvialis</i> and <i>Nannochloropsis</i> sp. – Theoretical model for the production of 1 kg of ME and 1.5–2.3 m ³ of biogas at STP – Assumes external CO ₂ and nutrient inputs – Assumes no internal energy recycling	[232]

ME = methyl esters; STP = standard temperature and pressure.

(mostly ammonium) [37].

Although algae growth on digestates from different feedstocks has been proposed by many authors [214–222], the use of algal digestate as source of nutrients for algae growth has been poorly studied. The first attempt into a closed loop for algae biomass and gas production through anaerobic digestion was reported by Golueke and Oswald [223]. They evaluated algae growth and biogas production through an innovative system composed by three components: an algae growth unit, an activated sludge and a digester. The feasibility of algae growth on algal digestate was demonstrated and the conversion of algae cells to methane reached an efficiency of approximately 66%.

Since this study, there are very few reports related to algal digestate as a fertilizer for algae growth. Björnsson et al. [224] evaluated *Sce-nedesmus* sp. AMDD growth on digestates from different feedstocks (algal biomass, co-digested swine manure/algal biomass, cow manure, swine manure) concluding that algal digestate has an advantage over the others due to its higher level of magnesium which is a main component of chlorophyll molecules and therefore an essential macro-nutrient for algae growth. Prajapati et al. [225] resumed Golueke and Oswald's work and proposed a closed loop process through the anaerobic digestion of the cyanobacteria *Chroococcus* sp. and subsequent recycling of nutrients in the digestate for algae growth of the same strain. With liquid digestate concentration of 30% the culture reached a biomass of 0.79 g L⁻¹. Their results showed that the proposed cycle is possible but that it needs further optimization to make it economically viable.

6.2. CO₂ recycling to boost algae productivity

A realistic possibility could be to burn the biogas onsite (e.g. for electricity production or to produce heat; e.g. for thermal pretreatment) and then use the CO₂ generated from this stream for improved micro-algae growth. This partly closes the loop for carbon, but CO₂ from the atmosphere will still be required as carbon is continuously removed from the system as biodiesel. Anaerobic digestion also directly generates CO₂ whose concentration in biogas is around 30–40% [200]. If methane is required in a pure form, for example for the production of chemicals [200], a cheap way to scrubbing methane could be to use microalgae cultures for biogas purification. At the same time the supplementation of CO₂ to the cultures could improve the productivity. However, its effectiveness is debatable due to the possible methane losses during the purification process.

The use of CO₂ from anaerobic digestion to purify biogas and support algae growth has been evaluated by only a few authors. The first approximation reported in the literature was done by Travieso et al. [227] who improved the productivity of *Arthrospira* sp. in around 2–5 times by using the CO₂ generated by the anaerobic digestion of molasses from a sugar refinery. Besides the benefits for algae cultivation, this experiment showed to be efficient in gas purification by increasing methane concentration from 55–77% to 88–97%. Another study developed by Heubeck et al. [228] showed that the supplementation of CO₂ in a high rate microalgae pond for wastewater treatment increased algae production and nutrient assimilation. Similarly, Doušková et al. [226] evaluated the use of CO₂ from anaerobic digestion of agricultural waste for cultivation of *Chlorella vulgaris* BELJ. The algae growth rate was similar when consuming biogas and when supplemented with a mixture of air and food-grade carbon dioxide, proving that the raw biogas could be used as carbon source for algae growth. The authors highlight two main advantages of using biogas as a source of carbon dioxide for algae growth: a reduction in the production costs and a final biomass free of harmful compounds that can arise in flue gases.

7. Energy balance and economic considerations

Economic and energy balances in microalgal production depend on strain selection, cultivation systems (e.g. photobioreactors or open ponds), growing conditions, end products (e.g. biodiesel and biogas), the market value of products and co-products, and production technologies (e.g. addition of CO₂, dewatering) [44]. Several studies show that coupling biodiesel and biogas systems can increase the economic and environmental feasibility of biofuel production operations, leading to more CO₂ savings as biogas can create the energy required for the production of biodiesel [44,45,165,171,229,230]. As a result, when coupling both systems, biodiesel production costs and CO₂ carbon emissions can decrease up to 33% and 75% respectively, [229], leading to energy output increases up to 40% [231].

Table 4 shows positive energy balances when both systems are integrated through nutrient, CO₂ and electricity recycling [44,45], and negative energy balances when using external nutrient and energy sources mostly derived from fossil fuels [232].

The feasibility of this coupling is dependent on the selected algal strain, which is expected to have high lipid contents in order to achieve large biodiesel yields [55], while high protein and carbohydrate contents increase biogas production [41,44]. High cell wall

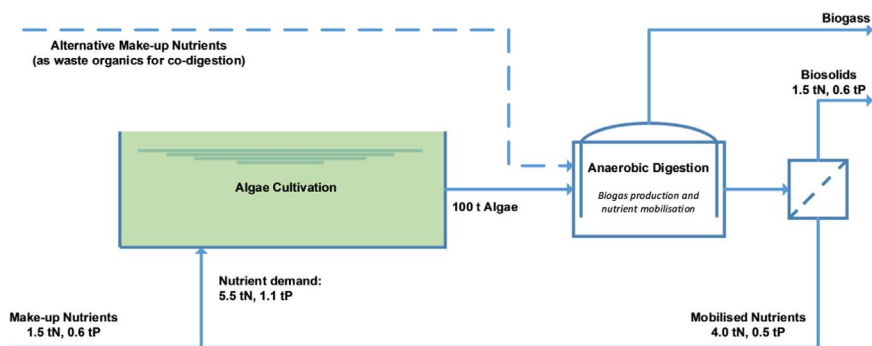


Fig. 2. Flow diagram of nutrient allocations based on a 100 ha microalgae farm used to produce biogas described by Collet et al. [165]. The model is based on a daily productivity of 25 t *C. vulgaris* day⁻¹ and 72% and 42% of nitrogen and phosphorous recovery are assumed.

biodegradability, low cell wall protein content and low sodium content strains are also desirable in order to increase the overall energy production when coupling biodiesel and biogas production systems [43].

Higher energy efficiencies and economic returns can be achieved through the optimization of energy intensive processes (e.g. during cultivation and oil extraction). Furthermore, nutrient recycling and the development of carbon sequestration loops could help in increasing the overall cost-effectiveness of the coupled system [232]. Nutrients recycled through anaerobic digestion could reduce upstream energy demand (i.e. fertilizers) in around 43–66% for nitrogen and in 20–39% for phosphorus [40]. As a result, there could be a need for an external source of nutrients in order to supplement the products of anaerobic digestion, including fresh fertilizer (make-up nutrients) or organic wastes for co-digestion, although in lower proportions (Fig. 2).

In relation to carbon sequestration, a coupled system for biodiesel and biogas production can achieve 50% of carbon recycling, leading to enhanced carbon efficiencies and economic benefits. This is because the other 50% of carbon is removed with the lipids that are used for biodiesel production [41]. This would equate to a reduction of the cultivation costs by 4% and a further 3.6% if methane were used for electricity generation in a closed looped system for biodiesel and biogas production. Even a reduction in CO₂ usage of 10% could achieve cost reductions greater than those achieved by a complete recycling of potassium, phosphate, and nitrogen [233].

Of the processes involved in microalgal production systems, harvesting is one of the most energy and cost intensive [234]. Cost-effective primary dewatering can be achieved by settling or flocculation, but this is species dependent and the addition of flocculants adds more costs and may inhibit anaerobic digestion [235]. Secondary dewatering usually requires the use of machinery such as a centrifuge, rotary press, or belt filter, which carry a very high capital expenditure and require a prohibitively large amount of electricity to run [44,229]. The highest improvement that could be made to the dewatering process would be adopting an adequate wet oil extraction technique [236]. Since wet algae sludge is appropriate for anaerobic digestion, this would reduce dewatering for the whole system to a case of merely settling, improving energy balances and reducing production costs [232,237].

8. Conclusions

An integrated microalgae system for biodiesel and biogas production is a promising alternative for reducing costly inputs and energy intensive processes, increasing the overall efficiency and the cost-effectiveness of biofuel generation technologies. Through anaerobic digestion, nutrients, CO₂ and energy are recycled, resulting in a closed loop for biofuel production which maximizes the generation of energy through biomass transformation, while significantly reducing production costs. The by-products of biodiesel and biogas production are valuable inputs for algae cultivation and anaerobic digestion, which substantially minimizes economic and energy demands. In order to optimize the process, it is important to choose species with high lipid and protein contents, gravity-assisted settling abilities, as well as thin

cell walls that facilitates lipid extraction and algae biodegradability. Some of the described pre-treatments significantly improve the digestibility by breaking the cells, which facilitates lipid extraction and anaerobic digestion. Besides, pre-treatments can improve percentages of nutrient mineralization which in general are too low to guarantee a complete efficiency of the closed nutrient loop. Because a high percentage of phosphorous is immobilized in biosolids, it is important to prevent pH increases higher than 8 in order to avoid struvite precipitation. Similarly, methanogenesis is pH sensitive. Additionally, mechanical disruption of digestate can help to increase available nutrients in the liquid phase for further microalgae growth. Finally, for minimal costs on harvesting and dewatering processes, it is suggested harvesting by sedimentation and lipid extraction on wet biomass.

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References

- [1] Martin MA. First generation biofuels compete. *New Biotechnol* 2010;27:596–608.
- [2] de Vries SC, van de Ven GW, van Ittersum MK, Giller KE. Resource use efficiency and environmental performance of nine major biofuel crops, processed by first-generation conversion techniques. *Biomass Bioenergy* 2010;34:588–601.
- [3] Castanheira EG, Grisoli R, Freire F, Pecora V, Coelho ST. Environmental sustainability of biodiesel in Brazil. *Energy Policy* 2014;65:680–91.
- [4] Naik S, Goud VV, Rout PK, Dalai AK. Production of first and second generation biofuels: a comprehensive review. *Renew Sustain Energy Rev* 2010;14:578–97.
- [5] Sims RE, Mabee W, Saddler JN, Taylor M. An overview of second generation biofuel technologies. *Bioresour Technol* 2010;101:1570–80.
- [6] Fargione J, Hill J, Tilman D, Polasky S, Hawthorne P. Land clearing and the biofuel carbon debt. *Science* 2008;319:1235–8.
- [7] Antizar-Ladislao B, Turrión-Gómez JL. Second-generation biofuels and local bioenergy systems. *Biofuels Bioprod Bioref* 2008;2:455–69.
- [8] Carriquiry MA, Du X, Timilsina GR. Second generation biofuels: economics and policies. *Energy Policy* 2011;39:4222–34.
- [9] Schulze ED, Körner C, Law BE, Haberl H, Luysaert S. Large-scale bioenergy from additional harvest of forest biomass is neither sustainable nor greenhouse gas neutral. *GCB Bioenergy* 2012;4:611–6.
- [10] Dauber J, Jones MB, Stout JC. The impact of biomass crop cultivation on temperate biodiversity. *Gcb Bioenergy* 2010;2:289–309.
- [11] Correa DF, Beyer HL, Possingham HP, Thomas-Hall SR, Schenk PM. Biodiversity impacts of bioenergy production: Microalgae vs. first generation biofuels. *Renew Sustain Energy Rev* 2017;74:1131–46.
- [12] Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C, et al. Second generation biofuels: high-efficiency microalgae for biodiesel production. *BioEnergy Res* 2008;1:20–43.
- [13] Chisti Y. Biodiesel from microalgae. *Biotechnol Adv* 2007;25:294–306.
- [14] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. *Renew Sustain Energy Rev* 2010;14:217–32.
- [15] Brennan L, Owende P. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew Sustain Energy Rev* 2010;14:557–77.
- [16] Ho S-H, Huang S-W, Chen C-Y, Hasunuma T, Kondo A, Chang J-S. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. *Bioresour Technol* 2013;135:191–8.

- [17] Harun R, Danquah MK. Influence of acid pre-treatment on microalgal biomass for bioethanol production. *Process Biochem* 2011;46:304–9.
- [18] Harun R, Jason W, Cherrington T, Danquah MK. Exploring alkaline pre-treatment of microalgal biomass for bioethanol production. *Appl Energy* 2011;88:3464–7.
- [19] Harun R, Danquah MK, Forde GM. Microalgal biomass as a fermentation feedstock for bioethanol production. *J Chem Technol Biotechnol* 2010;85:199–203.
- [20] Guldhe A, Singh B, Rawat I, Ramluckan K, Bux F. Efficacy of drying and cell disruption techniques on lipid recovery from microalgae for biodiesel production. *Fuel* 2014;128:46–52.
- [21] Lee J-Y, Yoo C, Jun S-Y, Ahn C-Y, Oh H-M. Comparison of several methods for effective lipid extraction from microalgae. *Bioresour Technol* 2010;101:S75–7.
- [22] Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chem Eng Process: Process Intensif* 2009;48:1146–51.
- [23] Johnson MB, Wen Z. Production of biodiesel fuel from the microalga *Schizochytrium limacinum* by direct transesterification of algal biomass. *Energy Fuels* 2009;23:5179–83.
- [24] Mandal S, Mallick N. Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. *Appl Microbiol Biotechnol* 2009;84:281–91.
- [25] Li X, Xu H, Wu Q. Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. *Biotechnol Bioeng* 2007;98:764–71.
- [26] Miao X, Wu Q. Biodiesel production from heterotrophic microalgal oil. *Bioresour Technol* 2006;97:841–6.
- [27] Prajapati SK, Kaushik P, Malik A, Vijay VK. Phycoremediation and biogas potential of native algal isolates from soil and wastewater. *Bioresour Technol* 2013;135:232–8.
- [28] Mussgnug JH, Klassen V, Schlüter A, Kruse O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J Biotechnol* 2010;150:51–6.
- [29] Vergara-Fernández A, Vargas G, Alarcón N, Velasco A. Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. *Biomass Bioenergy* 2008;32:338–44.
- [30] Yen H-W, Brune DE. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresour Technol* 2007;98:130–4.
- [31] Righoni-Stern S, Rismondo R, Szpyrkowicz L, Zilio-Grandi F, Vigato P. Anaerobic digestion of nitrophilic algal biomass from the Venice Lagoon. *Biomass* 1990;23:179–99.
- [32] Samson R, Leduy A. Biogas production from anaerobic digestion of *Spirulina maxima* algal biomass. *Biotechnol Bioeng* 1982;24:1919–24.
- [33] Beer LL, Boyd ES, Peters JW, Posewitz MC. Engineering algae for biohydrogen and biofuel production. *Curr Opin Biotechnol* 2009;20:264–71.
- [34] Laurinavichene TV, Tolstygina IV, Galiulina RR, Ghirardi ML, Seibert M, Tsygankov AA. Dilution methods to deprive *Chlamydomonas reinhardtii* cultures of sulfur for subsequent hydrogen photoproduction. *Int J Hydrog Energy* 2002;27:1245–9.
- [35] Tsygankov A, Fedorov A, Kosourov S, Rao K. Hydrogen production by cyanobacteria in an automated outdoor photobioreactor under aerobic conditions. *Biotechnol Bioeng* 2002;80:777–83.
- [36] Tsygankov A, Serebryakova L, Rao K, Hall D. Acetylene reduction and hydrogen photoproduction by wild-type and mutant strains of *Anabaena* at different CO₂ and O₂ concentrations. *FEMS Microbiol Lett* 1998;167:13–7.
- [37] Zhang Y, Kendall A, Yuan J. A comparison of on-site nutrient and energy recycling technologies in algal oil production. *Resour Conserv Recycl* 2014;88:13–20.
- [38] Alzate M, Muñoz R, Rogalla F, Fdz-Polanco F, Pérez-Elvira S. Biochemical methane potential of microalgal biomass after lipid extraction. *Chem Eng J* 2014;243:405–10.
- [39] Ramos-Suárez JL, Carreras N. Use of microalgae residues for biogas production. *Chem Eng J* 2014;242:86–95.
- [40] Keymer P, Ruffell I, Pratt S, Lant P. High pressure thermal hydrolysis as pre-treatment to increase the methane yield during anaerobic digestion of microalgae. *Bioresour Technol* 2013;131:128–33.
- [41] Zhu L. The combined production of ethanol and biogas from microalgal residuals to sustain microalgal biodiesel: a theoretical evaluation. *Biofuels Bioprod Bioref* 2014;8:7–15.
- [42] Ehimen E, Sun Z, Carrington C, Birch E, Eaton-Rye J. Anaerobic digestion of microalgal residues resulting from the biodiesel production process. *Appl Energy* 2011;88:3454–63.
- [43] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnol Adv* 2009;27:409–16.
- [44] Sapci Z, Morken J. The effect of algae species on biodiesel and biogas production observed by using a data model combines algae cultivation with an anaerobic digestion (ACAD) and a biodiesel process. *Energy Convers Manag* 2014;79:519–24.
- [45] Morken J, Sapci Z, Strømme JET. Modeling of biodiesel production in algae cultivation with anaerobic digestion (ACAD). *Energy Policy* 2013;60:98–105.
- [46] Chowdhury R, Viamajala S, Gerlach R. Reduction of environmental and energy footprint of microalgal biodiesel production through material and energy integration. *Bioresour Technol* 2012;108:102–11.
- [47] Sze P. A biology of the algae. Boston, USA: WCB/McGraw-Hill; 1998.
- [48] Barsanti L, Gualtieri P. Algae: anatomy, biochemistry, and biotechnology. Boca Raton, USA: CRC press; 2014.
- [49] Le Rouzic B, Bertru G. Phytoplankton community growth in enrichment bioassays: possible role of the nutrient intracellular pools. *Acta Oecologica* 1997;18:121–33.
- [50] Hecky RE, Kilham P. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. *Limnol Oceanogr* 1988;33:796–822.
- [51] Sigee DC. Freshwater microbiology. Chichester, UK: Wiley Online Library; 2005.
- [52] Lagus A. Role of nutrients in regulation of the phytoplankton community in the Archipelago Sea, northern Baltic Sea; 2009.
- [53] Healey F. Inorganic nutrient uptake and deficiency in algae. *Crit Rev Microbiol* 1973;3:69–113.
- [54] Grobbelaar JU. Algal nutrition—mineral nutrition. Handbook of microalgal culture: biotechnology and applied phycology. 2004. p. 95–115.
- [55] Wiley PE, Campbell JE, McKuin B. Production of biodiesel and biogas from algae: a review of process train options. *Water Environ Res* 2011;83:326–38.
- [56] Milledge JJ, Heaven S. A review of the harvesting of micro-algae for biofuel production. *Rev Environ Sci Bio/Technol* 2013;12:165–78.
- [57] Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *J Renew Sustain Energy* 2010;2:012701.
- [58] Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, et al. Biodiesel from algae: challenges and prospects. *Curr Opin Biotechnol* 2010;21:277–86.
- [59] Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, et al. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J* 2008;54:621–39.
- [60] Khozin-Goldberg I, Cohen Z. The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water euglenophyte *Monodus subterraneus*. *Phytochemistry* 2006;67:696–701.
- [61] Zhila NO, Kalacheva GS, Volova TG. Influence of nitrogen deficiency on biochemical composition of the green alga *Botryococcus*. *J Appl Phycol* 2005;17:309–15.
- [62] Guihéneuf F, Fouqueray M, Mimouni V, Ulmann L, Jacqueline B, Tremblin G. Effect of UV stress on the fatty acid and lipid class composition in two marine microalgae *Pavlova lutheri* (Pavlovophyceae) and *Odontella aurita* (Bacillariophyceae). *J Appl Phycol* 2010;22:629–38.
- [63] Skerratt JH, Davidson AD, Nichols PD, McMeekin TA. Effect of UV-B on lipid content of three Antarctic marine phytoplankton. *Phytochemistry* 1998;49:999–1007.
- [64] Li Y, Qin JG. Comparison of growth and lipid content in three *Botryococcus braunii* strains. *J Appl Phycol* 2005;17:551–6.
- [65] Kalacheva G, Zhila N, Volova T, Gladyshev M. The effect of temperature on the lipid composition of the green alga *Botryococcus*. *Microbiology* 2002;71:286–93.
- [66] Fábregas J, Maseda A, Domínguez A, Otero A. The cell composition of *Nannochloropsis* sp. changes under different irradiances in semicontinuous culture. *World J Microbiol Biotechnol* 2004;20:31–5.
- [67] Chiu S-Y, Kao C-Y, Tsai M-T, Ong S-C, Chen C-H, Lin C-S. Lipid accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂ aeration. *Bioresour Technol* 2009;100:833–8.
- [68] Gordillo FJ, Goutx M, Figueroa FL, Niell FX. Effects of light intensity, CO₂ and nitrogen supply on lipid class composition of *Dunaliella viridis*. *J Appl Phycol* 1998;10:135–44.
- [69] Tsuzuki M, Ohnuma E, Sato N, Takaku T, Kawaguchi A. Effects of CO₂ concentration during growth on fatty acid composition in microalgae. *Plant Physiol* 1990;93:851–6.
- [70] Ruangsomborn S. Effect of light, nutrient, cultivation time and salinity on lipid production of newly isolated strain of the green microalga, *Botryococcus braunii* KMITL 2. *Bioresour Technol* 2012;109:261–5.
- [71] Takagi M, Yoshida T. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. *J Biosci Bioeng* 2006;101:223–6.
- [72] Liang Y, Sarkany N, Cui Y. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. *Biotechnol Lett* 2009;31:1043–9.
- [73] Oh SH, Han JG, Kim Y, Ha JH, Kim SS, Jeong MH, et al. Lipid production in *Porphyridium cruentum* grown under different culture conditions. *J Biosci Bioeng* 2009;108:429–34.
- [74] Sharma KK, Schuhmann H, Schenk PM. High lipid induction in microalgae for biodiesel production. *Energies* 2012;5:1532–53.
- [75] Cheng Y, Zhou W, Gao C, Lan K, Gao Y, Wu Q. Biodiesel production from Jerusalem artichoke (*Helianthus Tuberosus* L.) tuber by heterotrophic microalgae *Chlorella protothecoides*. *J Chem Technol Biotechnol* 2009;84:777–81.
- [76] Xu H, Miao X, Wu Q. High quality biodiesel production from a microalga *Chlorella protothecoides* by heterotrophic growth in fermenters. *J Biotechnol* 2006;126:499–507.
- [77] Shen Y, Pei Z, Yuan W, Mao E. Effect of nitrogen and extraction method on algae lipid yield. *Int J Agric Biol Eng* 2009;2:51–7.
- [78] Pal D, Khozin-Goldberg I, Cohen Z, Boussiba S. The effect of light, salinity, and nitrogen availability on lipid production by *Nannochloropsis* sp. *Appl Microbiol Biotechnol* 2011;90:1429–41.
- [79] Liu J, Huang J, Sun Z, Zhong Y, Jiang Y, Chen F. Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: assessment of algal oils for biodiesel production. *Bioresour Technol* 2011;102:106–10.
- [80] Ho S-H, Chang J-S, Lai Y-Y, Chen C-NN. Achieving high lipid productivity of a thermotolerant microalga *Desmodesmus* sp. F2 by optimizing environmental factors and nutrient conditions. *Bioresour Technol* 2014;156: [108–16].
- [81] Nascimento IA, Marques SSI, Cabanelas ITD, Pereira SA, Druzian JI, de Souza CO, et al. Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profiles as selective criteria. *BioEnergy Res* 2013;6:1–13.
- [82] Taher H, Al-Zuhair S, Al-Marzouqi AH, Haik Y, Farid M. Effective extraction of

- microalgae lipids from wet biomass for biodiesel production. *Biomass Bioenergy* 2014;66:159–67.
- [83] Li Y, Horsman M, Wang B, Wu N, Lan CQ. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Appl Microbiol Biotechnol* 2008;81:629–36.
- [84] Hsieh C-H, Wu W-T. Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. *Bioresour Technol* 2009;100:3921–6.
- [85] Ho S-H, Chen W-M, Chang J-S. *Scenedesmus obliquus* CNW-N as a potential candidate for CO₂ mitigation and biodiesel production. *Bioresour Technol* 2010;101:8725–30.
- [86] Yeh K-L, Chen C-Y, Chang J-S. pH-stat photoheterotrophic cultivation of indigenous *Chlorella vulgaris* ESP-31 for biomass and lipid production using acetic acid as the carbon source. *Biochem Eng J* 2012;64:1–7.
- [87] Singh P, Guldhe A, Kumari S, Rawat I, Bux F. Investigation of combined effect of nitrogen, phosphorus and iron on lipid productivity of microalgae *Ankistrodesmus falcatus* KJ671624 using response surface methodology. *Biochem Eng J* 2015;94:22–9.
- [88] Yeh K-L, Chang J-S. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31. *Bioresour Technol* 2012;105:120–7.
- [89] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol Bioeng* 2009;102:100–12.
- [90] Praveenkumar R, Shameera K, Mahalakshmi G, Akbarsha MA, Thajuddin N. Influence of nutrient deprivations on lipid accumulation in a dominant indigenous microalga *Chlorella* sp., BUM11008: evaluation for biodiesel production. *Biomass Bioenergy* 2012;37:60–6.
- [91] Gouveia L, Oliveira AC. Microalgae as a raw material for biofuels production. *J Ind Microbiol Biotechnol* 2009;36:269–74.
- [92] Meher L, Sagar DV, Naik S. Technical aspects of biodiesel production by transesterification—a review. *Renew Sustain Energy Rev* 2006;10:248–68.
- [93] Leung DY, Wu X, Leung M. A review on biodiesel production using catalyzed transesterification. *Appl Energy* 2010;87:1083–95.
- [94] Hara M. Environmentally benign production of biodiesel using heterogeneous catalysts. *ChemSusChem* 2009;2:129–35.
- [95] Cooney M, Young G, Nagle N. Extraction of bio-oils from microalgae. *Sep Purif Rev* 2009;38:291–325.
- [96] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–7.
- [97] Catchpole O, Tallon S, Eltringham W, Grey J, Fenton K, Vagi E, et al. The extraction and fractionation of specialty lipids using near critical fluids. *J Supercrit Fluids* 2009;47:591–7.
- [98] Sahena F, Zaidul I, Jinap S, Karim A, Abbas K, Norulaini N, et al. Application of supercritical CO₂ in lipid extraction – a review. *J Food Eng* 2009;95:240–53.
- [99] Barreiro DL, Prins W, Ronse F, Brillman W. Hydrothermal liquefaction (HTL) of microalgae for biofuel production: state of the art review and future prospects. *Biomass Bioenergy* 2013;53:113–27.
- [100] Toor SS, Rosendahl L, Rudolf A. Hydrothermal liquefaction of biomass: a review of subcritical water technologies. *Energy* 2011;36:2328–42.
- [101] Iqbal J, Theegala C. Microwave assisted lipid extraction from microalgae using biodiesel as co-solvent. *Algal Res* 2013;2:34–42.
- [102] Balasubramanian S, Allen JD, Kanitkar A, Boldor D. Oil extraction from *Scenedesmus obliquus* using a continuous microwave system—design, optimization, and quality characterization. *Bioresour Technol* 2011;102:3396–403.
- [103] Cho H-S, Oh Y-K, Park S-C, Lee J-W, Park J-Y. Effects of enzymatic hydrolysis on lipid extraction from *Chlorella vulgaris*. *Renew Energy* 2013;54:156–60.
- [104] Fu C-C, Hung T-C, Chen J-Y, Su C-H, Wu W-T. Hydrolysis of microalgae cell walls for production of reducing sugar and lipid extraction. *Bioresour Technol* 2010;101:8750–4.
- [105] Yoo G, Park W-K, Kim CW, Choi Y-E, Yang J-W. Direct lipid extraction from wet *Chlamydomonas reinhardtii* biomass using osmotic shock. *Bioresour Technol* 2012;123:717–22.
- [106] Bai X, Naghdi FG, Ye L, Lant P, Pratt S. Enhanced lipid extraction from algae using free nitrous acid pretreatment. *Bioresour Technol* 2014;159:36–40.
- [107] Araujo GS, Matos LJ, Fernandes JO, Cartaxo SJ, Gonçalves LR, Fernandes FA, et al. Extraction of lipids from microalgae by ultrasound application: prospection of the optimal extraction method. *Ultrason Sonochem* 2013;20:95–8.
- [108] Adam F, Abert-Vian M, Peltier G, Chemat F. “Solvent-free” ultrasound-assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. *Bioresour Technol* 2012;124:457–65.
- [109] Zbinden MDA, Sturm BS, Nord RD, Carey WJ, Moore D, Shinogle H, et al. Pulsed electric field (PEF) as an intensification pretreatment for greener solvent lipid extraction from microalgae. *Biotechnol Bioeng* 2013;110:1605–15.
- [110] Goettel M, Eing C, Gusbeth C, Straessner R, Frey W. Pulsed electric field assisted extraction of intracellular valuable from microalgae. *Algal Res* 2013;2:401–8.
- [111] Garcia Alba L, Torri C, Samori C, van der Spek J, Fabbri D, Kersten SR, et al. Hydrothermal treatment (HTT) of microalgae: evaluation of the process as conversion method in an algae biorefinery concept. *Energy Fuels* 2011;26:642–57.
- [112] Mercer P, Armenta RE. Developments in oil extraction from microalgae. *Eur J Lipid Sci Technol* 2011;113:539–47.
- [113] Ziemiński K, Frac M. Methane fermentation process as anaerobic digestion of biomass: transformations, stages and microorganisms. *Afr J Biotechnol* 2014;11:4127–39.
- [114] Kwietniewska E, Tys J. Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation. *Renew Sustain Energy Rev* 2014;34:491–500.
- [115] Uggetti E, Sialve B, Trably E, Steyer JP. Integrating microalgae production with anaerobic digestion: a biorefinery approach. *Biofuels Bioprod Bioref* 2014;8:516–29.
- [116] Osorio F, Torres J. Biogas purification from anaerobic digestion in a wastewater treatment plant for biofuel production. *Renew Energy* 2009;34:2164–71.
- [117] Gelegenis J, Georgakakis D, Angelidaki I, Christopoulou N, Goumenaki M. Optimization of biogas production from olive-oil mill wastewater, by codigesting with diluted poultry-manure. *Appl Energy* 2007;84:646–63.
- [118] Bougrier C, Delgenes J-P, Carrere H. Combination of thermal treatments and anaerobic digestion to reduce sewage sludge quantity and improve biogas yield. *Process Saf Environ Prot* 2006;84:280–4.
- [119] Demirel GN, Duran M, Güven E, Ugurlu Ö, Tezel U, Ergüder TH. Anaerobic treatability and biogas production potential studies of different agro-industrial wastewaters in Turkey. *Biodegradation* 2000;11:401–5.
- [120] Comino E, Riggio VA, Rosso M. Biogas production by anaerobic co-digestion of cattle slurry and cheese whey. *Bioresour Technol* 2012;114:46–53.
- [121] Clemens J, Trimborn M, Weiland P, Amon B. Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry. *Agric Ecosyst Environ* 2006;112:171–7.
- [122] Wulf S, Jäger P, Döhler H. Balancing of greenhouse gas emissions and economic efficiency for biogas-production through anaerobic co-fermentation of slurry with organic waste. *Agric Ecosyst Environ* 2006;112:178–85.
- [123] Lallai A, Mura G, Onnis N. The effects of certain antibiotics on biogas production in the anaerobic digestion of pig waste slurry. *Bioresour Technol* 2002;82:205–8.
- [124] El-Mashad HM, Zhang R. Biogas production from co-digestion of dairy manure and food waste. *Bioresour Technol* 2010;101:4021–8.
- [125] Ahn HK, Smith M, Kondrad S, White J. Evaluation of biogas production potential by dry anaerobic digestion of switchgrass–animal manure mixtures. *Appl Biochem Biotechnol* 2010;160:965–75.
- [126] Chae K, Jang A, Yim S, Kim IS. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresour Technol* 2008;99:1–6.
- [127] Hansen KH, Angelidaki I, Ahring BK. Anaerobic digestion of swine manure: inhibition by ammonia. *Water Res* 1998;32:5–12.
- [128] Banks CJ, Chesshire M, Heaven S, Arnold R. Anaerobic digestion of source-segregated domestic food waste: performance assessment by mass and energy balance. *Bioresour Technol* 2011;102:612–20.
- [129] Cho JK, Park SC, Chang HN. Biochemical methane potential and solid state anaerobic digestion of Korean food wastes. *Bioresour Technol* 1995;52:245–53.
- [130] Viswanath P, Devi SS, Nand K. Anaerobic digestion of fruit and vegetable processing wastes for biogas production. *Bioresour Technol* 1992;40:43–8.
- [131] Bruni E, Jensen AP, Pedersen ES, Angelidaki I. Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. *Appl Energy* 2010;87:2212–7.
- [132] Gerin PA, Vlieghe F, Jossart J-M. Energy and CO₂ balance of maize and grass as energy crops for anaerobic digestion. *Bioresour Technol* 2008;99:2620–7.
- [133] Leubuh M, Liu F, Heuwinkel H, Gronauer A. Biogas production from mono-digestion of maize silage-long-term process stability and requirements. *Water Sci Technol* 2008;58:1645.
- [134] Amon T, Amon B, Kryvoruchko V, Zollitsch W, Mayer K, Gruber L. Biogas production from maize and dairy cattle manure—influence of biomass composition on the methane yield. *Agric Ecosyst Environ* 2007;118:173–82.
- [135] Strik D, Domnanovich A, Holubar P. A pH-based control of ammonia in biogas during anaerobic digestion of artificial pig manure and maize silage. *Process Biochem* 2006;41:1235–8.
- [136] Xie S, Frost J, Lawlor P, Wu G, Zhan X. Effects of thermo-chemical pre-treatment of grass silage on methane production by anaerobic digestion. *Bioresour Technol* 2011;102:8748–55.
- [137] Kaparaju P, Serrano M, Thomsen AB, Kongjan P, Angelidaki I. Bioethanol, bio-hydrogen and biogas production from wheat straw in a biorefinery concept. *Bioresour Technol* 2009;100:2562–8.
- [138] He Y, Pang Y, Liu Y, Li X, Wang K. Physicochemical characterization of rice straw pretreated with sodium hydroxide in the solid state for enhancing biogas production. *Energy Fuels* 2008;22:2775–81.
- [139] Zhang R, Zhang Z. Biogasification of rice straw with an anaerobic-phased solids digester system. *Bioresour Technol* 1999;68:235–45.
- [140] Müller H, Trösch W. Screening of white-rot fungi for biological pretreatment of wheat straw for biogas production. *Appl Microbiol Biotechnol* 1986;24:180–5.
- [141] Lehtomäki A, Huttunen S, Lehtinen T, Rintala J. Anaerobic digestion of grass silage in batch leach bed processes for methane production. *Bioresour Technol* 2008;99:3267–78.
- [142] Xie S, Lawlor P, Frost J, Hu Z, Zhan X. Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. *Bioresour Technol* 2011;102:5728–33.
- [143] Mähner P, Heiermann M, Linke B. Batch- and semi-continuous biogas production from different grass species. *Agric Eng Int: CIGR J* 2005.
- [144] Golueke CG, Oswald WJ, Gotaas HB. Anaerobic digestion of algae. *Appl Microbiol* 1957;5:47.
- [145] Ramos-Suárez JL, Martínez A, Carreras N. Optimization of the digestion process of *Scenedesmus* sp. and *Opuntia maxima* for biogas production. *Energy Convers Manag* 2014;88:1263–70.
- [146] Prajapati SK, Malik A, Vijay VK. Comparative evaluation of biomass production and biorefinery generation potential of *Chlorella* spp. through anaerobic digestion. *Appl Energy* 2014;114:790–7.
- [147] Alcántara C, García-Encina PA, Muñoz R. Evaluation of mass and energy balances in the integrated microalgae growth-anaerobic digestion process. *Chem Eng J* 2013;221:238–46.

- [148] Frigon J-C, Matteau-Lebrun F, Abdou RH, McGinn PJ, O'Leary SJ, Guioir SR. Screening microalgae strains for their productivity in methane following anaerobic digestion. *Appl Energy* 2013;108:100–7.
- [149] Mairet F, Bernard O, Cameron E, Ras M, Lardon L, Steyer JP, et al. Three-reaction model for the anaerobic digestion of microalgae. *Biotechnol Bioeng* 2012;109:415–25.
- [150] Ras M, Lardon L, Bruno S, Bernet N, Steyer J-P. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. *Bioresour Technol* 2011;102:200–6.
- [151] Marzano C-MADS, Legros A, Naveau H, Nyns E-J. Biomethanation of the Marine Algae *Tetraselmis*. *Int J Sol Energy* 1982;1:263–72.
- [152] Yuan X, Shi X, Zhang D, Qiu Y, Guo R, Wang L. Biogas production and microcystin biodegradation in anaerobic digestion of blue algae. *Energy Environ Sci* 2011;4:1511–5.
- [153] Yan Q, Zhao M, Miao H, Ruan W, Song R. Coupling of the hydrogen and polyhydroxyalkanoates (PHA) production through anaerobic digestion from Taihu blue algae. *Bioresour Technol* 2010;101:4508–12.
- [154] Zeng S, Yuan X, Shi X, Qiu Y. Effect of inoculum/substrate ratio on methane yield and orthophosphate release from anaerobic digestion of *Microcystis* spp. *J Hazard Mater* 2010;178:89–93.
- [155] Grimm P, Risse JM, Cholewa D, Müller JM, Beshay U, Friehs K, et al. Applicability of *Euglena gracilis* for biorefineries demonstrated by the production of α -tocopherol and paracylon followed by anaerobic digestion. *J Biotechnol* 2015.
- [156] Bogen C, Klassen V, Wichmann J, La Russa M, Doebe A, Grundmann M, et al. Identification of *Monoraphidium contortum* as a promising species for liquid biofuel production. *Bioresour Technol* 2013;133:622–6.
- [157] Zamalloa C, Boon N, Verstraete W. Anaerobic digestibility of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* under mesophilic and thermophilic conditions. *Appl Energy* 2012;92:733–8.
- [158] Haiduc AG, Brandenberger M, Suquet S, Vogel F, Bernier-Latmani R, Ludwig C. SunChem: an integrated process for the hydrothermal production of methane from microalgae and CO₂ mitigation. *J Appl Phycol* 2009;21:529–41.
- [159] Vivekanand V, Eijssink VG, Horn SJ. Biogas production from the brown seaweed *Saccharina latissima*: thermal pretreatment and codigestion with wheat straw. *J Appl Phycol* 2012;24:1295–301.
- [160] Briand X, Morand P. Anaerobic digestion of *Ulva* sp. 1. Relationship between *Ulva* composition and methanisation. *J Appl Phycol* 1997;9:511–24.
- [161] Chynoweth DP, Klass DL, Ghosh S. Biomethanation of giant brown kelp *Macrocystis pyrifera*. In: Proceedings of the energy from biomass and wastes symposium. Washington, DC; 1978. p. 229.
- [162] González-Fernández C, Sialve B, Bernet N, Steyer J. Thermal pretreatment to improve methane production of *Scenedesmus* biomass. *Biomass Bioenergy* 2012;40:105–11.
- [163] Hernández ES, Córdoba LT. Anaerobic digestion of *Chlorella vulgaris* for energy production. *Resour Conserv Recycl* 1993;9:127–32.
- [164] González-Fernández C, Sialve B, Bernet N, Steyer J. Comparison of ultrasound and thermal pretreatment of *Scenedesmus* biomass on methane production. *Bioresour Technol* 2012;110:610–6.
- [165] Collet P, Hélias A, Lardon L, Ras M, Goy R-A, Steyer J-P. Life-cycle assessment of microalgae culture coupled to biogas production. *Bioresour Technol* 2011;102:207–14.
- [166] Yang Z, Guo R, Xu X, Fan X, Luo S. Hydrogen and methane production from lipid-extracted microalgal biomass residues. *Int J Hydrog Energy* 2011;36:3465–70.
- [167] Polakovičová G, Kušník P, Nagyová S, Mikulec J. Process integration of algae production and anaerobic digestion. *Chem Eng* 2012:29.
- [168] Stevenson RJ, Bothwell ML, Lowe RL. Algal ecology: freshwater benthic ecosystems. San Diego, USA: Academic Press; 1996.
- [169] Lee RE. Phycology. Cambridge, UK: Cambridge University Press; 2008.
- [170] Foree EG, McCarty PL. Anaerobic decomposition of algae. *Environ Sci Technol* 1970;4:842–9.
- [171] Ward A, Lewis D, Green F. Anaerobic digestion of algae biomass: a review. *Algal Res* 2014;5:204–14.
- [172] Kayhanian M. Performance of a high-solids anaerobic digestion process under various ammonia concentrations. *J Chem Technol Biotechnol* 1994;59:349–52.
- [173] Liu T, Sung S. Ammonia inhibition on thermophilic aceticlastic methanogens. *Water Sci Technol* 2002;45:113–20.
- [174] Zhong W, Zhang Z, Luo Y, Qiao W, Xiao M, Zhang M. Biogas productivity by co-digesting Taihu blue algae with corn straw as an external carbon source. *Bioresour Technol* 2012;114:281–6.
- [175] Chen Y, Cheng JJ, Creamer KS. Inhibition of anaerobic digestion process: a review. *Bioresour Technol* 2008;99:4044–64.
- [176] Feijoo G, Soto M, Mendez R, Lema JM. Sodium inhibition in the anaerobic digestion process: antagonism and adaptation phenomena. *Enzym Microb Technol* 1995;17:180–8.
- [177] Omil F, Méndez R, Lema JM. Anaerobic treatment of saline wastewaters under high sulphide and ammonia content. *Bioresour Technol* 1995;54:269–78.
- [178] McCarty PL, McKinney RE. Salt toxicity in anaerobic digestion. *J (Water Pollut Control Fed)* 1961:399–415.
- [179] Patel G, Roth L. Effect of sodium chloride on growth and methane production of methanogens. *Can J Microbiol* 1977;23:893–7.
- [180] Kugelman IJ, Toxicity Chin K. synergism, and antagonism in anaerobic waste treatment processes. *Adv Chem Ser* 1971;105:55–90.
- [181] Rinzema A, van Lier J, Lettinga G. Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzym Microb Technol* 1988;10:24–32.
- [182] Vanegas C, Bartlett J. Green energy from marine algae: biogas production and composition from the anaerobic digestion of Irish seaweed species. *Environ Technol* 2013;34:2277–83.
- [183] Bird KT, Chynoweth DP, Jerger DE. Effects of marine algal proximate composition on methane yields. *J Appl Phycol* 1990;2:207–13.
- [184] Hanssen JF, Indergaard M, Østgaard K, Bævre OA, Pedersen TA, Jensen A. Anaerobic digestion of *Laminaria* spp. and *Ascophyllum nodosum* and application of end products. *Biomass* 1987;14:1–13.
- [185] Passos F, Uggetti E, Carrère H, Ferrer I. Pretreatment of microalgae to improve biogas production: a review. *Bioresour Technol* 2014;172:403–12.
- [186] Appels L, Impe JV, Dewil R. Oxidizing agents and organic solvents as pretreatment for anaerobic digestion. *Biogas production: pretreatment methods in anaerobic digestion*. 2012. p. 199–214. New Jersey, USA.
- [187] Carrère H, Dumas C, Battimelli A, Batstone D, Delgenès J, Steyer J, et al. Pretreatment methods to improve sludge anaerobic degradability: a review. *J Hazard Mater* 2010;183:1–15.
- [188] Alzate M, Muñoz R, Rogalla F, Fdz-Polanco F, Pérez-Elvira S. Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. *Bioresour Technol* 2012;123:488–94.
- [189] Schwede S, Rehman Z-U, Gerber M, Theiss C, Span R. Effects of thermal pretreatment on anaerobic digestion of *Nannochloropsis salina* biomass. *Bioresour Technol* 2013;143:505–11.
- [190] Passos F, Ferrer I. Microalgae conversion to biogas: thermal pretreatment contribution on net energy production. *Environ Sci Technol* 2014;48:1717–8.
- [191] Passos F, García J, Ferrer I. Impact of low temperature pretreatment on the anaerobic digestion of microalgal biomass. *Bioresour Technol* 2013;138:79–86.
- [192] Passos F, Astals S, Ferrer I. Anaerobic digestion of microalgal biomass after ultrasound pretreatment. *Waste Manag* 2014;34:2098–103.
- [193] Marin J, Kennedy KJ, Eskicioglu C. Effect of microwave irradiation on anaerobic degradability of model kitchen waste. *Waste Manag* 2010;30:1772–9.
- [194] Lee K, Chantarasakdakul P, Kim D, Kong M, Park KY. Ultrasound pretreatment of filamentous algal biomass for enhanced biogas production. *Waste Manag* 2014;34:1035–40.
- [195] Park KY, Kwon J, Chantarasakdakul P, Lee K, Cha HY. Anaerobic digestion of microalgal biomass with ultrasonic disintegration. *Int Biodeterior Biodegrad* 2013;85:598–602.
- [196] Dejoye C, Vian MA, Lumia G, Bouscarle C, Charton F, Chemat F. Combined extraction processes of lipid from *Chlorella vulgaris* microalgae: microwave prior to supercritical carbon dioxide extraction. *Int J Mol Sci* 2011;12:9332–41.
- [197] Passos F, Solé M, García J, Ferrer I. Biogas production from microalgae grown in wastewater: effect of microwave pretreatment. *Appl Energy* 2013;108:168–75.
- [198] Passos F, Hernandez-Marine M, García J, Ferrer I. Long-term anaerobic digestion of microalgae grown in HRAP for wastewater treatment. Effect of microwave pretreatment. *Water Res* 2014;49:351–9.
- [199] Schwede S, Kowalczyk A, Gerber M, Span R. Influence of different cell disruption techniques on mono digestion of algal biomass. In: Proceedings of the world renewable energy congress. Linköping, Sweden; 2011. p. 8–13.
- [200] Bohutskyi P, Bouwer E. Biogas production from algae and cyanobacteria through anaerobic digestion: a review, analysis, and research needs. *Adv Biofuels Bioprod* 2013;8:73–975.
- [201] Bohutskyi P, Betenbaugh MJ, Bouwer EJ. The effects of alternative pretreatment strategies on anaerobic digestion and methane production from different algal strains. *Bioresour Technol* 2014;155:366–72.
- [202] Holm-Nielsen JB, Al Seadi T, Oleskowicz-Popiel P. The future of anaerobic digestion and biogas utilization. *Bioresour Technol* 2009;100:5478–84.
- [203] Yilmazel Y, Demirer G. Removal and recovery of nutrients as struvite from anaerobic digestion residues of poultry manure. *Environ Technol* 2011;32:783–94.
- [204] Massé D, Croteau F, Masse L. The fate of crop nutrients during digestion of swine manure in psychrophilic anaerobic sequencing batch reactors. *Bioresour Technol* 2007;98:2819–23.
- [205] Bauer A, Mayr H, Hopfner-Sixt K, Amon T. Detailed monitoring of two biogas plants and mechanical solid–liquid separation of fermentation residues. *J Biotechnol* 2009;142:56–63.
- [206] Balasubramanian P, Bai RK. Evaluation of nutrient recovery after anaerobic digestion of cattle dung in a family size biogas plant. *Biomass Bioenergy* 1992;3:377–80.
- [207] Field JA, Caldwell JS, Jeyanayagam S, Reneau Jr. RB, Kroontje W, Collins Jr. ER. Fertilizer recovery from anaerobic digesters. *Trans ASAE* 1984;27:1871–6.
- [208] Güngör K, Karthikeyan K. Phosphorus forms and extractability in dairy manure: a case study for Wisconsin on-farm anaerobic digesters. *Bioresour Technol* 2008;99:425–36.
- [209] Marcihac C, Sialve B, Pourcher A-M, Ziebal C, Bernet N, Béline F. Control of nitrogen behaviour by phosphate concentration during microalgal-bacterial cultivation using digestate. *Bioresour Technol* 2015;175:224–30.
- [210] Ihara I, Toyoda K, Watanabe T, Umetsu K. Nitrogen and energy balances of a combined anaerobic digestion and electrochemical oxidation process for dairy manure management. *Anim Prod Sci* 2008;48:208–12.
- [211] Alburquerque JA, de la Fuente C, Ferrer-Costa A, Carrasco L, Cegarra J, Abad M, et al. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. *Biomass Bioenergy* 2012;40:181–9.
- [212] Möller K, Müller T. Effects of anaerobic digestion on digestate nutrient availability and crop growth: a review. *Eng Life Sci* 2012;12:242–57.
- [213] Arthurson V. Closing the global energy and nutrient cycles through application of biogas residue to agricultural land–potential benefits and drawback. *Energies* 2009;2:226–42.
- [214] Budiyo IS, Sumardiono S, Sasongko SB. Production of *Spirulina platensis* biomass using digested vinasse as cultivation medium. *Trends Appl Sci Res* 2014;9:93–102.
- [215] Cicci A, Bravi M. Production of the freshwater microalgae *Scenedesmus dimorphus*

- and *Arthrospira platensis* by using cattle digestate. Chem Eng 2014;38.
- [216] Ji F, Liu Y, Hao R, Li G, Zhou Y, Dong R. Biomass production and nutrients removal by a new microalgae strain *Desmodesmus* sp. in anaerobic digestion wastewater. Bioresour Technol 2014;161:200–7.
- [217] Cai T, Park SY, Racharaks R, Li Y. Cultivation of *Nannochloropsis salina* using anaerobic digestion effluent as a nutrient source for biofuel production. Appl Energy 2013;108:486–92.
- [218] Franchino M, Comino E, Bona F, Riggio VA. Growth of three microalgae strains and nutrient removal from an agro-zootechnical digestate. Chemosphere 2013;92:738–44.
- [219] Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J, et al. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. Bioresour Technol 2010;101:2623–8.
- [220] Phang S, Miah M, Yeoh B, Hashim M. Spirulina cultivation in digested sago starch factory wastewater. J Appl Phycol 2000;12:395–400.
- [221] Blier R, Laliberte G, De la Noüe J. Tertiary treatment of cheese factory anaerobic effluent with *Phormidium bohneri* and *Micractinium pusillum*. Bioresour Technol 1995;52:151–5.
- [222] Olguín E, Hernández B, Araus A, Camacho R, González R, Ramírez M, et al. Simultaneous high-biomass protein production and nutrient removal using *Spirulina maxima* in sea water supplemented with anaerobic effluents. World J Microbiol Biotechnol 1994;10:576–8.
- [223] Golueke CG, Oswald WJ. Biological conversion of light energy to the chemical energy of methane. Appl Microbiol 1959;7:219–27.
- [224] Björnsson WJ, Nicol RW, Dickinson KE, McGinn PJ. Anaerobic digestates are useful nutrient sources for microalgae cultivation: functional coupling of energy and biomass production. J Appl Phycol 2013;25:1523–8.
- [225] Prajapati SK, Kumar P, Malik A, Vijay VK. Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: a closed loop bioenergy generation process. Bioresour Technol 2014;158:174–80.
- [226] Doušková I, Kaštánek F, Maléterová Y, Kaštánek P, Doucha J, Zachleder V. Utilization of distillery stillage for energy generation and concurrent production of valuable microalgal biomass in the sequence: biogas-cogeneration-microalgae-products. Energy Convers Manag 2010;51:606–11.
- [227] Travieso L, Sanchez E, Benitez F, Conde J. *Arthrospira* sp. intensive cultures for food and biogas purification. Biotechnol Lett 1993;15:1091–4.
- [228] Heubeck S, Craggs R, Shilton A. Influence of CO₂ scrubbing from biogas on the treatment performance of a high rate algal pond. Water Sci Technol 2007;55:193–200.
- [229] Harun R, Davidson M, Doyle M, Gopiraj R, Danquah M, Forde G. Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. Biomass Bioenergy 2011;35:741–7.
- [230] Lyko H, Deerberg G, Weidner E. Coupled production in biorefineries—combined use of biomass as a source of energy, fuels and materials. J Biotechnol 2009;142:78–86.
- [231] Bohutskyi P, Kula T, Kessler BA, Hong Y, Bouwer EJ, Betenbaugh MJ, et al. Mixed trophic state production process for microalgal biomass with high lipid content for generating biodiesel and biogas. BioEnergy Res 2014;7:1174–85.
- [232] Razon LF, Tan RR. Net energy analysis of the production of biodiesel and biogas from the microalgae: *Haematococcus pluvialis* and *Nannochloropsis*. Appl Energy 2011;88:3507–14.
- [233] Richardson JW, Johnson MD, Zhang X, Zemke P, Chen W, Hu Q. A financial assessment of two alternative cultivation systems and their contributions to algae biofuel economic viability. Algal Res 2014;4:96–104.
- [234] Slade R, Bauen A. Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. Biomass Bioenergy 2013;53:29–38.
- [235] Gossett JM, McCarty PL, Wilson JC, Evans DS. Anaerobic digestion of sludge from chemical treatment. J (Water Pollut Control Fed) 1978:533–42.
- [236] Naghdi F Ghasemi, González González LM, Chan W, Schenk PM. Progress on lipid extraction from wet algal biomass for biodiesel production. Microb Biotechnol 2016;9:718–26.
- [237] Lardon L, Hélias A, Sialve B, Steyer J-P, Bernard O. Life-cycle assessment of biodiesel production from microalgae. Environ Sci Technol 2009;43:6475–81.